

TABLE 1

Study no. 44/009

Validation for the determination of MDA in human plasma:  
Intra-assay precision and accuracy of quality control sample data in water

Replicate	QC 0.4 $\mu\text{mol/L}$ (LLOQ QC)	QC 1 $\mu\text{mol/L}$ (LoQC)	QC 4 $\mu\text{mol/L}$ (MeQC)	QC 8 $\mu\text{mol/L}$ (HiQC)
	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )
1	0.39	0.92	3.78	7.65
2	0.43	0.95	3.91	7.50
3	0.44	1.05	4.03	7.87
4	0.45	1.05	3.72	8.37
5	NR*	NR*	4.44	8.13
6	0.68*	1.62*	4.28	8.37
Mean ( $\mu\text{mol/L}$ )	0.43	0.99	4.03	7.98
SD (n-1)	0.026	0.068	0.284	0.368
Precision (%)	6.0	6.9	7.0	4.6
Accuracy (%)	107.5	99.0	100.8	99.8

NR = No result

\* two samples possibly transferred to same tube in error, result not included in statistics

TABLE 1a

Validation for the determination of MDA in human plasma:  
Intra-assay precision and accuracy of quality control sample data in plasma

Replicate	QC 1 $\mu\text{mol/L}$ (LoQC)	QC 4 $\mu\text{mol/L}$ (MeQC)	QC 8 $\mu\text{mol/L}$ (HiQC)
	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )
1	0.95	3.79	7.34
2	1.00	3.87	7.34
3	0.97	4.05	7.58
4	0.92	3.92	7.63
5	0.91	3.88	7.57
6	0.90	3.88	7.82
Mean ( $\mu\text{mol/L}$ )	0.94	3.90	7.55
SD (n-1)	0.039	0.086	0.184
Precision (%)	4.1	2.2	2.4
Accuracy (%)	94.0	97.5	94.4

TABLE 2

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Validation for the determination of MDA in human plasma:  
Inter-assay precision and accuracy of quality control sample data in water

Batch	Replicate	QC 0.4 $\mu\text{mol/L}$ (LLOQ QC)	QC 1 $\mu\text{mol/L}$ (LoQC)	QC 4 $\mu\text{mol/L}$ (MeQC)	QC 8 $\mu\text{mol/L}$ (HiQC)
		Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )
PVAL01RI	1	0.39	0.92	3.78	7.65
	2	0.43	0.95	3.91	7.50
	3	0.44	1.05	4.03	7.87
	4	0.45	1.05	3.72	8.37
	5	NR*	NR*	4.44	8.13
	6	0.68*	1.62*	4.28	8.37
PVAL02I	1	0.36	0.91	3.70	7.33
	2	0.39	0.96	3.89	7.65
	3	0.34	1.03	3.98	7.99
	4	0.41	1.04	4.12	8.21
	5	0.42	1.05	4.14	8.14
	6	0.42	1.04	4.35	8.78

NR = No result

\* two samples possibly transferred to same tube in error, result not included in statistics

TABLE 2a

Validation for the determination of MDA in human plasma:  
Inter-assay precision and accuracy of quality control sample data in water

Batch	Replicate	QC 1 $\mu\text{mol/L}$ (LoQC)	QC 4 $\mu\text{mol/L}$ (MeQC)	QC 8 $\mu\text{mol/L}$ (HiQC)
		Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )
PVAL01RI	1	0.95	3.79	7.34
	2	1.00	3.87	7.34
	3	0.97	4.05	7.58
	4	0.92	3.92	7.63
	5	0.91	3.88	7.57
	6	0.90	3.88	7.82
PVAL02I	1	0.84	3.56	7.02
	2	0.92	3.78	7.42
	3	0.94	3.83	7.55
	4	0.99	3.80	7.74
	5	0.90	3.94	7.75
	6	0.89	4.11	8.10

TABLE 2 (continued)

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Validation for the determination of MDA in human plasma:  
Inter-assay precision and accuracy of quality control sample data in water

Batch	Replicate	QC 0.4 $\mu\text{mol/L}$ (LLOQ QC)	QC 1 $\mu\text{mol/L}$ (LoQC)	QC 4 $\mu\text{mol/L}$ (MeQC)	QC 8 $\mu\text{mol/L}$ (HiQC)
		Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )
PVAL03	1	-	0.90	3.71	7.34
	2	-	0.94	3.78	7.33
	3	-	0.97	3.88	7.96
	4	-	1.04	4.15	8.26
	5	-	1.06	4.27	8.50
	6	-	1.09	4.28	8.38
PVAL05	1	-	0.88	3.74	7.55
	2	-	0.97	3.90	7.91
	3	-	0.93	3.95	8.04
	4	-	0.97	4.07	8.11
	5	-	0.99	4.25	8.63
	6	-	1.03	4.36	8.64
Mean ( $\mu\text{mol/L}$ )		0.41	0.99	4.03	8.03
Standard deviation (n-1)		0.035	0.061	0.233	0.432
Precision (%)		8.5	6.2	5.8	5.4
Accuracy (%)		102.5	99.0	100.8	100.4
n		10	22	24	24

TABLE 2a (continued)

Validation for the determination of MDA in human plasma:  
Inter-assay precision and accuracy of quality control sample data in plasma

Batch	Replicate	QC 1 $\mu\text{mol/L}$ (LoQC)	QC 4 $\mu\text{mol/L}$ (MeQC)	QC 8 $\mu\text{mol/L}$ (HiQC)
		Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )	Observed concentration ( $\mu\text{mol/L}$ )
PVAL03	1	0.99	3.59	6.85
	2	0.95	3.59	7.07
	3	0.92	3.90	7.38
	4	1.03	3.88	7.71
	5	0.99	4.15	7.71
	6	0.99	3.95	7.92
PVAL05	1	0.85	3.54	6.89
	2	0.92	3.68	7.15
	3	0.96	3.74	7.23
	4	0.94	3.82	7.56
	5	1.00	4.04	7.91
	6	1.10	3.94	7.68
Mean ( $\mu\text{mol/L}$ )		0.95	3.84	7.50
Standard deviation (n-1)		0.058	0.167	0.334
Precision (%)		6.1	4.3	4.5
Accuracy (%)		95.0	96.0	93.8
n		24	24	24

TABLE 3

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Validation for the determination of MDA in human plasma:  
Inter-assay accuracy and precision of calibration standard data

Batch	Back-calculated concentrations ( $\mu\text{mol/L}$ )									Curve parameters		
	Calibration level ( $\mu\text{mol/L}$ )									Gradient (m)	Intercept (c)	Coefficient of determination ( $r^2$ )
	0.4	0.6	1	2	3	5	7.5	9	10			
PVAL01RI	0.42	0.65	0.88	1.66 PF	3.03	4.63	7.71	8.89	10.28	8.55660E 04	1.02740E 04	0.99742
PVAL02I	0.46	0.60	0.95	1.87	3.06	4.34	7.65	9.71	9.85	5.64649E 04	9.73083E 03	0.99147
PVAL03	0.35	0.67	1.04	1.88	3.10	4.86	7.74	9.74	9.12	6.66945E 04	4.47859E 03	0.98721
PVAL05	0.35	0.64	1.05	1.97	3.17	5.01	7.53	7.79	11.00	6.29813E 04	7.25626E 03	0.97809
Mean ( $\mu\text{mol/L}$ )	0.40	0.64	0.98	1.91	3.09	4.71	7.66	9.03	10.06	-	-	-
Standard deviation (n-1)	0.054	0.029	0.080	0.055	0.061	0.292	0.093	0.917	0.787	-	-	-
Precision (%)	13.5	4.5	8.2	2.9	2.0	6.2	1.2	10.2	7.8	-	-	-
Accuracy (%)	100.0	106.7	98.0	95.5	103.0	94.2	102.1	100.3	100.6	-	-	-

PF – Poor fit, calibration standard outside acceptance criteria

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TABLE 4

Validation for the determination of MDA in human plasma:  
Individual unspiked plasma samples  
(endogenous levels)

Matrix sample number	Back calculated concentration (umol/L)	Mean back calculated concentrations (umol/L) (CV%)
01H408	0.57	0.6
01H409	0.62	(10.0)
01H410	0.61	
01H411	0.67	
01H412	0.57	
01H319	0.48	
01H182	0.66	
01H183	0.58	
01H184	0.66	
01H318	0.70	

TABLE 5

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**Validation for the determination of MDA in human plasma:**  
**Recovery of MDA from human plasma**

Quality control level ( $\mu\text{mol/L}$ )	Replicate	Extracted QCs. Conc ( $\mu\text{mol/L}$ )	Mean peak area (CV%)	Reference QCs. Conc ( $\mu\text{mol/L}$ )	Mean peak area (CV%)	Recovery (%)
1	1	0.99	1.0	0.90	1.0	100.0
	2	0.95	(4.0)	0.94	(7.0)	
	3	0.92		0.97		
	4	1.03		1.04		
	5	0.99		1.06		
	6	0.99		1.09		
4	1	3.59	3.8	3.71	4.0	94.7
	2	3.59	(5.7)	3.78	(6.3)	
	3	3.90		3.88		
	4	3.88		4.15		
	5	4.15		4.27		
	6	3.95		4.28		
8	1	6.85	7.4	7.34	8.0	92.5
	2	7.07	(5.6)	7.33	(6.5)	
	3	7.38		7.96		
	4	7.71		8.26		
	5	7.71		8.50		
	6	7.92		8.38		
Mean recovery (%)						95.7

TABLE 6

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Validation for the determination of MDA in human plasma:  
Room temperature stability of MDA in human plasma  
(24 hours)

Quality control level ( $\mu\text{mol/L}$ )	Replicate	Baseline QC samples		QC samples stored for 24 hours prior to extraction		Difference from baseline (%)
		Observed concentration ( $\mu\text{mol/L}$ )	Mean ( $\mu\text{mol/L}$ ) (CV%)	Observed concentration ( $\mu\text{mol/L}$ )	Mean ( $\mu\text{mol/L}$ ) (CV%)	
1	1	0.99	0.98 (3.9)	0.87	0.89 (4.4)	-9.2
	2	0.95		0.84		
	3	0.92		0.95		
	4	1.03		0.89		
	5	0.99		0.89		
	6	0.99		0.88		
8	1	6.85	7.44 (5.6)	6.02	6.17 (2.3)	-17.1
	2	7.07		6.00		
	3	7.38		6.11		
	4	7.71		6.22		
	5	7.71		6.28		
	6	7.92		6.36		

TABLE 6a

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Validation for the determination of MDA in human plasma:  
Room temperature stability of MDA in human plasma  
(18 hours)

Quality control level ( $\mu\text{mol/L}$ )	Replicate	Baseline QC samples		QC samples stored for 18 hours prior to extraction		Difference from baseline (%)
		Observed concentration ( $\mu\text{mol/L}$ )	Mean ( $\mu\text{mol/L}$ ) (CV%)	Observed concentration ( $\mu\text{mol/L}$ )	Mean ( $\mu\text{mol/L}$ ) (CV%)	
1	1	0.85	0.96 (8.8)	0.87	0.90 (1.8)	-6.2
	2	0.92		0.88		
	3	0.96		0.91		
	4	0.94		0.92		
	5	1.00		0.91		
	6	1.10		0.92		
8	1	6.89	7.40 (5.1)	6.24	6.58 (4.9)	-11.1
	2	7.15		6.24		
	3	7.23		6.49		
	4	7.56		6.66		
	5	7.91		6.77		
	6	7.68		7.10		



TABLE 6b

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Validation for the determination of MDA in human plasma:  
Room temperature stability of MDA in human plasma  
(12 hours)

Quality control level ( $\mu\text{mol/L}$ )	Replicate	Baseline QC samples		QC samples stored for 12 hours prior to extraction		Difference from baseline (%)
		Observed concentration ( $\mu\text{mol/L}$ )	Mean ( $\mu\text{mol/L}$ ) (CV%)	Observed concentration ( $\mu\text{mol/L}$ )	Mean ( $\mu\text{mol/L}$ ) (CV%)	
1	1	0.85	0.96 (8.8)	0.86	0.90 (6.3)	-6.2
	2	0.92		0.85		
	3	0.96		0.87		
	4	0.94		0.89		
	5	1.00		0.97		
	6	1.10		0.97		
8	1	6.89	7.40 (5.1)	6.47	6.83 (4.3)	-7.7
	2	7.15		6.58		
	3	7.23		6.59		
	4	7.56		7.10		
	5	7.91		7.03		
	6	7.68		7.19		

TABLE 7

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Validation for the determination of MDA in human plasma:  
Freeze/thaw stability of MDA in human plasma

Quality control level ( $\mu\text{mol/L}$ )	Replicate	Baseline QC samples		QC samples subjected to 3 additional freeze/thaw cycles prior to extraction		Difference from baseline (%)
		Observed concentration ( $\mu\text{mol/L}$ )	Mean ( $\mu\text{mol/L}$ ) (CV%)	Observed concentration ( $\mu\text{mol/L}$ )	Mean ( $\mu\text{mol/L}$ ) (CV%)	
1	1	0.84	0.91 (5.5)	0.81	0.88 (4.2)	-3.3
	2	0.92		0.88		
	3	0.94		0.87		
	4	0.99		0.87		
	5	0.90		0.92		
	6	0.89		0.90		
8	1	7.02	7.60 (4.8)	6.66	7.39 (5.7)	-2.8
	2	7.42		7.22		
	3	7.55		7.36		
	4	7.74		7.53		
	5	7.75		7.71		
	6	8.10		7.85		

TABLE 8

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Validation for the determination of MDA in human plasma:  
Stability of extracted QC samples from plasma, stored refrigerated for 3 days

Quality control level (μmol/L)	Replicate	Stored extracted QC samples		
		Observed concentration (μmol/L)	Mean (μmol/L) (CV%)	Difference from nominal concentration (%)
1	1		#DIV/0!	#DIV/0!
	2		#DIV/0!	
	3			
	4			
	5			
	6			
4	1		#DIV/0!	#DIV/0!
	2		#DIV/0!	
	3			
	4			
	5			
	6			
8	1		#DIV/0!	#DIV/0!
	2		#DIV/0!	
	3			
	4			
	5			
	6			

TABLE 8a

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Validation for the determination of MDA in human plasma:  
Stability of extracted QC samples from water, stored refrigerated for 3 days

Quality control level (μmol/L)	Replicate	Stored extracted QC samples		
		Observed concentration (μmol/L)	Mean (μmol/L) (CV%)	Difference from nominal concentration (%)
1	1		#DIV/0!	#DIV/0!
	2		#DIV/0!	
	3			
	4			
	5			
	6			
4	1		#DIV/0!	#DIV/0!
	2		#DIV/0!	
	3			
	4			
	5			
	6			
8	1		#DIV/0!	#DIV/0!
	2		#DIV/0!	
	3			
	4			
	5			
	6			

TABLE 9

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**Validation for the determination of MDA in human plasma:**  
**Stability of stock solutions stored at nominal 4°C for 29 days**

Date of stock solution preparation	Replicate	Peak absorbance of stock solution	Mean peak absorbance value	% Difference of stored solution from new solution
13/08/01 (new)	1		#DIV/0!	#DIV/0!
	2			
	3			
16/07/01 (stored)	1		#DIV/0!	
	2			
	3			

TABLE 3

Study no. 44/009

Validation for the determination of MDA in human plasma:  
Response function batch accuracy and precision of calibration standard data

Matrix	Back-calculated concentrations ( $\mu\text{mol/L}$ )									Curve parameters		
	Calibration level ( $\mu\text{mol/L}$ )									Gradient (m)	Intercept (c)	Coefficient of determination ( $r^2$ )
	0.4	0.6	1	2	3	5	7.5	9	10			
Water	0.44	0.62	0.62 PF	1.86	2.81	4.75	7.42	9.19	10.41	5.60778E 04	5.970631E 03	0.99688
011H408	0.43	0.62	0.98	1.89	2.92	4.79	7.36	9.17	10.34	5.39809E 04	2.22627E 04	0.99806
011H409	0.40	0.65	0.99	1.91	2.94	4.81	7.32	9.04	10.45	5.38390E 04	2.85398E 04	0.99756
011H410	0.42	0.66	0.89	1.98	2.92	4.77	7.36	9.14	10.36	5.38928E 04	3.72385E 04	0.99789
011H411	0.44	0.62	0.94	1.86	2.94	5.01	7.36	8.96	10.37	5.37681E 04	3.54146E 04	0.99836
011H412	0.43	0.61	0.94	2.00	2.97	4.91	7.27	8.98	10.40	5.40267E 04	2.40573E 04	0.99803
011B319	0.43	0.60	0.97	1.90	3.93 PF	4.97	7.54	8.97	10.12	5.37025E 04	2.28820E 04	0.99974
Mean ( $\mu\text{mol/L}$ )	0.43	0.63	0.95	1.91	2.92	4.86	7.38	9.06	10.35	54184.0	-	-
Standard deviation (n-1)	0.014	0.021	0.037	0.055	0.055	0.104	0.086	0.100	0.108	842.72	-	-
Precision (%)	3.3	3.3	3.9	2.9	1.9	2.1	1.2	1.1	1.0	1.6	-	-
Accuracy (%)	107.5	105.0	95.0	95.5	97.3	97.2	98.4	100.7	103.5	-	-	-

PF = Poor fit, calibration standard outside acceptance criteria